**Title:** Standardization of foldscope in comparison with a microscope to identify E. coli for further use in field settings

**Short title:** Standardization of Foldscope for identification of E. coli

**Authors Name:**

1. Jeyakumar Angeline[1,2]
2. Juana Hatwik[1]
3. Swapnil Godbharle[1]
4. Bibek Raj Giri[1]

[1] Interdisciplinary School of Health Sciences, Savitribai Phule Pune University, Ganeshkhind-411007, Pune, Maharashtra, India

[2] Senior Research Associate, University of Johannesburg, South Africa

Citation:

**Abstract**

The study aimed to standardize foldscope for identification of E. coli using standard E. coli cultures. Cultures of E coli were prepared on nutrient agar medium, gram stained and images were captured using foldscope (140X) and microscope (40X x 10X). Images were recorded in duplicates for both tools. The total images in duplicates of E. coli were n=14 for each tool (n=28). Images were fed in Image –J software (Fiji) and cell count in specified area was calculated for both foldscope and microscope. Bootstrap quantile analysis was performed to assess difference between median values. Bootstrap sampling with thousand resample replacement showed no significant difference between the test and standard instrument (p=0.002). The clarity of the images and color of the stain through microscope was sharper compared to foldscope. However, variation in cell count between both instruments was minimal making it an ideal tool to be used in settings where laboratories are not available.

**Key words: Frugal technology, Foldscope, E. coli, Standardization**

## Introduction

Utilizing frugal technology to address public health issues, specifically infections have received much attention in the recent years. Diarrhea continues to be a major killer infection among children under five globally [i]. In India the prevalence of diarrhea is relatively high compared to other countries. E. coli is a causative organism for diarrhea among infants and children. In public health E. coli is an indicator organism that is often tested in water samples for contamination from biological pathogens. Indicator organisms are present in larger numbers, and can be easily identified and controlled by simple public health measures to prevent infections. WHO recognizes that 1.4 million child deaths from diarrhea could be prevented by providing safe drinking water [ii]. A preventive plan for diarrheal infections should essentially identify the presence of coliforms in water sources. The challenge in isolated, resource poor settings such as tribal regions includes minimal use of technology for their routine activities and poor infrastructure. This calls for a frugal tool, ideal for settings that lack optimal laboratory infrastructure. Frugal technology has immense scope in providing innovative strategies to bridge disparities in resource constrained settings.

Foldscopes are optical, origami microscopes suitable for various imaging modalities such as bright or dark field or fluorescence microscopy [iii], weighs 8.8g and has potential application in testing the presence of E. coli in drinking water sources in isolated regions. Foldscope has been standardized for screening *Schistosoma haematobium* [iv] and malarial parasites [v]. However, to utilize foldscope for identifying E. coli in drinking water sources it is essential to standardize the foldscope. This work therefore aims to standardize foldscope for identification of E. coli using standard E. coli cultures for further use in tribal settings.

## Materials and methods

### Standardization of foldscope

To standardize foldscope for identification of E. coli, preliminary procedures such as preparation of pure E. coli cultures and gram staining were performed in the laboratory. This was followed by recording, enumerating and comparing the images between the test and the standard instrument using appropriate software. The details of procedure and software used are explained below.

i. ***Preparation of E. coli cultures*:** Weighed 0.49gm of media (Luria broth), added 20 ml of distilled water, mixed thoroughly and divided into sterilized test tubes, autoclaved for 15 minutes at 120°C. To 1 ml of broth 20ul of E. coli pure strain was added and incubated at 37°C for 24 hours. Using a sterilized inoculating loop a full loop of E. coli culture from the broth was taken and spread over the 1st quadrant (approximately ¼ of the plate by close parallel streaks). The loop was sterilized and swept over the 1st spread; the plate was turned 90° and streaked without overlapping the previous streaks. The procedure was repeated twice after turning the plate 90°. The plates were inverted and incubated at 37°C for 24 hours.

ii. ***Gram staining*:** A sample of bacterial colony was removed from the plate cultures and stirred into 1ml saline. From this 20µl was pipetted for slide preparation. The smears were dried and heat fixed and smeared first with crystal violet, followed by Gram's iodine, with subsequent decolorization with ethyl alcohol. The slides were counterstained with safranin and dried. A set of 14 slides of standard E. coli cultures were observed and counted in duplicates (N=14x2=28).

iii. **Image capture and standardization of foldscope for enumeration of E. coli**

The E. coli smears on the slides were marked with a tip of a marker and the area close to the mark was observed both under the microscope and foldscope. All images were recorded in duplicates.

a. ***Digital image capture:*** Images of E. coli from the slides were captured by a digital camera using a Samsung J2 mobile phone. Images captured using foldscope (magnification of 140x) were compared with the images captured using a microscope (magnification of 400x).

b. ***Image J automated counting*:** Image J is a Java based free software downloadable from the National Institute of Health (NIH) website, US. Automated counting of the E. coli in Image J, involves threshold algorithms to distinguish the E. coli cells from the background. The threshold level is dependent on the algorithm selected to study E. coli. The algorithms selected were process, binary, outline. To set the counting threshold, the image was opened with the following commands: Process>Binary>Outline>Sharpen>Analyze. Each counted particle was outlined and numbered in a new window.

Bootstrap quantile statistics was performed to determine the difference between the median values obtained through the two instruments (i.e. microscope and foldscope).

**Results**

Images recorded using foldscope and microscope are provided in the supplementary file. Enumeration of E. coli was done in duplicates for both foldscope and microscope and average values are presented in Table 1. Bootstrap sampling with thousand resamples replacement showed no significant difference between the test and standard instrument (p=0.002).

**Table 1: Difference in median between E. coli count through microscope and foldscope**

| Pure E. Coli Sample | Mean duplicate E. coli count of test and standard instrument using Image J | | Bootstrap Analysis | | Significance |
|---|---|---|---|---|---|
| | Microscope (standard) | Foldscope (test) | Quantiles | Difference in median | |
| **1** | 62 | 54 | 2.5% CI | 0.04 | **p=0.002** |
| **2** | 62 | 52 | 97.5% CI | 0.13 | |
| **3** | 62 | 54 | | | |
| **4** | 86 | 76 | | | |
| **5** | 86 | 83 | | | |
| **6** | 86 | 74 | | | |
| **7** | 86 | 73 | | | |
| **8** | 86 | 80 | | | |
| **9** | 86 | 76 | | | |
| **10** | 86 | 82 | | | |
| **11** | 86 | 82 | | | |
| **12** | 86 | 78 | | | |
| **13** | 86 | 84 | | | |
| **14** | 86 | 81 | | | |

**Discussion**

Prevention of infections in resource poor settings requires smart, inexpensive tools, to enable screening in field setting. Public health screening when integrated with frugal technology offers the potential to carry science to areas unreached. Our preliminary effort, systematically evaluated the quality of images using quantitative measurements combining the technology of an origami microscope (foldscope) with a mobile phone and image J software [vi]. Statistical analysis of the median values showed no significant difference between the test and standard instrument, confirming foldscope as a tool for identifying standard E. coli. However, as observed in other studies in our images too, center of the image of E. coli through foldscope had better clarity compared to the edge. The use of spherical lenses in foldscope provides variation in the clarity of image, where clarity is higher in the center of the field and images appear blurred at the edge due to significant error [iii, iv] . Microscopic image capture has seen a paradigm shift after the use of mobile phones for recording images in the field of medicine and public health[vii]. It has found wide application in health research and other fields as well. However, in our study although mobile phone offered the ease of capturing E. coli images, the characteristic pink color of the E. coli after gram staining that is observed directly through the foldscope could not be retained in mobile images. Novel techniques of image processing using mobile phones have been tested to address this limitation [viii] . Addressing these limitations in this frugal tool offers major scope for field-based applications. Our work utilized Image J software to standardize the test tool with the standard light microscope. Other studies that have employed image J software for enumerating cells have proven it to be economical, standardized and reproducible compared to manual methods [ix].

In contrast to conventional microscopes, foldscope offers the advantage of ease of assembly, is economical and portable with good magnification, one that is ideal to bridge the community and lab in isolated settings. Improving the magnification of the lens will increase the utility of the tool especially in isolated settings both for research and public health screening. Training healthcare providers with the skills of assembling foldscopes and using it will enable testing of drinking water sources for contamination in isolated settings.

## Conclusion

The clarity of the images and color of the stain through microscope was sharper compared to foldscope. However, variation in cell count between both instruments was minimal making it an ideal tool to be used in settings where laboratories are not available.

**Acknowledgements:**

This work was supported by the Department of Biotechnology (DBT), Government of India with grant number BT/IN/Indo-US/Foldscope/39/2015. DBT had no role in the design, analysis or writing of this article.

Conflict of interest: The authors have no conflicts of interest to disclose.

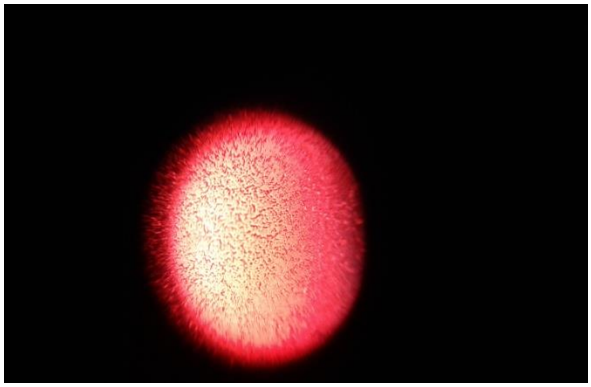

Image 1: Fluorescent image of E. coli under foldscope

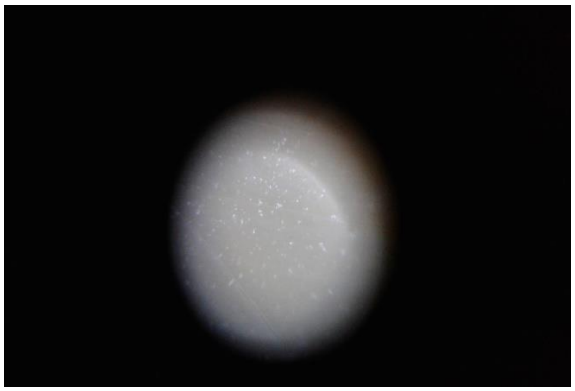

Image 2: Dark field image of E. coli under foldscope

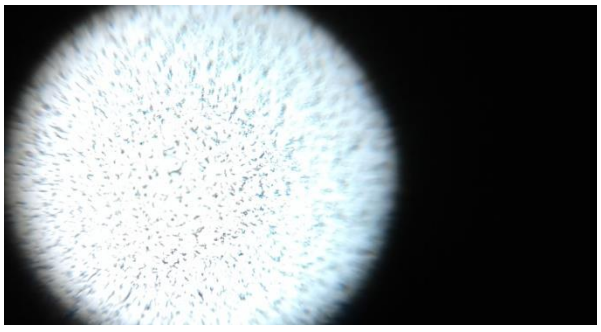

Image 3: Bright field image of E. coli under Foldscope

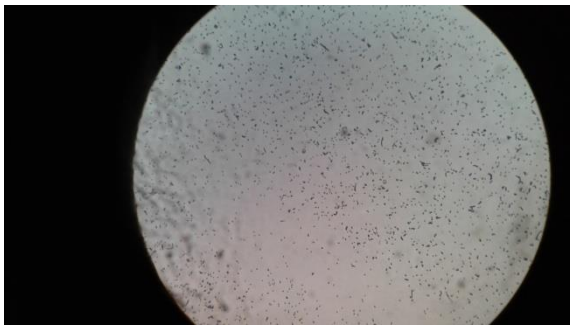

Image 4: Bright field image of E. coli under Microscope

## References


i. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SR, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018;18: 1211–1228.

ii. Annette Prüss-Üstün, Robert Bos, Fiona Gore, Jamie Bartram. Safer water, better health : costs, benefits and sustainability of interventions to protect and promote health. Geneva: World Health Organization; 2008. Available: https://apps.who.int/iris/bitstream/handle/10665/43840/9789241596435_eng.pdf;jsessionid=0A7C58CD462EF85A985CA21B6B5640F8?sequence=1

iii. Cybulski JS, Clements J, Prakash M. Foldscope: Origami-Based Paper Microscope. Martens L, editor. PLoS ONE. 2014;9: e98781. doi:10.1371/journal.pone.0098781

iv. Ephraim RKD, Keiser J, Duah E, Cybulski JS, Prakash M, D'Ambrosio MV, et al. Diagnosis of Schistosoma haematobium Infection with a Mobile Phone-Mounted Foldscope and a Reversed-Lens CellScope in Ghana. Am J Trop Med Hyg. 2015;92: 1253–1256. doi:10.4269/ajtmh.14-0741

v. Shailaja M, Jolitha A.B, Divya G. E, Ranjitha. A, Archana Preethi. R, Gowari Neelima, et al. Malaria parasite detection-Employability of foldscope in malarial diagnosis. Int J Adv Res Biol Sci. 2019;6.

vi. Rateni G, Dario P, Cavallo F. Smartphone-Based Food Diagnostic Technologies: A Review. Sensors. 2017;17: 1453. doi:10.3390/s17061453

vii. Dendere R, Myburg N, Douglas TS. A review of cellphone microscopy for disease detection: A REVIEW OF CELLPHONE MICROSCOPY FOR DISEASE DETECTION. J Microsc. 2015;260: 248–259. doi:10.1111/jmi.12307

viii. Skandarajah A, Reber CD, Switz NA, Fletcher DA. Quantitative imaging with a mobile phone microscope. PloS One. 2014;9: e96906.

ix. Siritantikorn S, Jintaworn S, Noisakran S, Suputtamongkol Y, Paris DH, Blacksell SD. Application of ImageJ program to the enumeration of Orientia tsutsugamushi organisms cultured in vitro. Trans R Soc Trop Med Hyg. 2012;106: 632–635. doi:10.1016/j.trstmh.2012.05.004